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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/031,695	01/23/2002	Bernhard Hauer	51241	8284

26474 7590 03/18/2005

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EXAMINER	
PAK, YONG D	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 03/18/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/031,695	HAUER ET AL.	
	Examiner	Art Unit	
	Yong D Pak	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 November 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12 and 14-17* is/are pending in the application.

4a) Of the above claim(s) *1-11* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *12 and 14-17* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *12/20/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This application is a 371 of PCT/EP00/07252.

The amendment filed on November 29, 2004, amending claims 12 and 14-15 anc canceling claim 13, has been entered.

Claims 1-12 and 14-17 are pending. Claims 1-11 are withdrawn. Claims 12 and 14-17 are under consideration.

Election/Restrictions

Claims 1-11 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 8.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on December 20, 2004 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Response to Arguments

Applicant's amendment and arguments filed on November 29, 2004, have been fully considered and are deemed to be persuasive to overcome the rejections previously

applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 12 and claims 14-17 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 recites the phrase "isolating resulting hydroxylated product from the medium" and "subterminally hydroxylated aliphatic carboxylic acids". These phrases are not clear to the Examiner. Modified P450 monooxygenases hydroxylate both at the terminal and subterminal positions of a carboxylic acid. Therefore, a) it is not clear to the Examiner how applicants distinguish or direct the enzyme to make only subterminally hydroxylated products and b) it is not clear to the examiner the steps of isolating subterminally hydroxylated carboxylic acids from terminally hydroxylated carboxylic acids. In the context of the above, Examiner takes the position that these claims are incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: steps in isolating subterminally hydroxylated carboxylic acids from terminally hydroxylated carboxylic

acids and directing the enzyme towards making only "subterminally hydroxylated aliphatic carboxylic acids".

Claim 12 and claims 14-17 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 12, the phrase "shows an altered substrate profile" is unclear. It is not clear to the Examiner if the modified enzyme has an increased or decreased "substrate profile". The metes and bounds of the above phrase is not clear, rendering the claim indefinite. It is also not clear to the Examiner how an enzyme "shows" its substrate profile.

Claims 12 and 14-15 and claims 14-17 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 a2) recites the phrase " said monooxygenase". It is unclear to the Examiner whether the phrase is referring to the modified monooxygenase of 12 a1) or to any other monooxygenase. This ambiguity arises because step 12 a1) is mainly drawn to culturing a transformant and the claim never refers to a "modified enzyme" distinctly in the preceding part of the claim. Examiner has interpreted that the phrase refers to the modified enzyme and urges applicants to clarify in response to the Office Action.

Claims 12 and 14 and claims 15-17 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 12 and 14 recite the phrase "C_8-C_{12}-carboxylic acid derivatives". The metes and bounds of this phrase is not clear to the Examiner. Literally, the term "derivative" means a substance that can be made from another substance. Therefore, it is not clear to the Examiner either from the specification or form the claims as to what applicants mean by the above phrase.

Claims 14-15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 14-15 recite the phrase "monooxygenase (SEQ ID NO:2) used" or "monooxygenase (SEQ ID NO:2) employed". The phrases are confusing because SEQ ID NO:2 is the wildtype P450 monooxygenase of *Bacillus megaterium*. The claim as written does not make it clear whether the enzyme used has SEQ ID NO:2 or whether the enzyme having an amino acid sequence SEQ ID NO;2 is modified further. For example, it is not clear whether residue "F87V" corresponds specifically to SEQ ID NO:2 or any monooxygenase. Applicants are urged not to use SEQ ID NO: in parenthesis as it leads to ambiguity.

Claims 14-15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 14-15 recited groups of amino acid substitutions, such as in claim 14 part d) "F87A L188K A74G". It is not clear if the amino acid substitutions in these groups is in the alternative or is all-inclusive.

Claim 16 and claim 17 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 16 recites the limitation "the reaction" in line 1. There is insufficient antecedent basis for this limitation in the claim. This limitation "the reaction" has no antecedence in claim 12 from which claim 16 depends from. Applicants need to amend claim 16 to recite "culturing/incubation" in place of "the reaction".

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 12 and 16-17 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of enzymatic production of specific subterminally hydroxylated aliphatic carboxylic acids by using a modified cytochrome P450 monooxygenase with SEQ ID NO:2 having single or multiple mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 with 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA as substrates, does not reasonably provide enablement for A) a method for the production of any subterminally hydroxylated aliphatic carboxylic acids and B) wherein said method encompasses the use of any modified cytochrome P450 monooxygenase. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. (See rejection of "C_8-C_{12}-carboxylic acid derivatives" under 35 U.S.C. 112, 2nd paragraph).

Factors to be considered in determining whether undue experimentation is required, are summarized in In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 12 and 16-17 are drawn to a method for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids with a modified cytochrome P450 monooxygenase having an altered substrate binding region. Therefore, the claims

encompass a method for the production of any or all subterminally hydroxylated aliphatic carboxylic acids using recombinants, variants and mutants of any P450 monooxygenase and having any alterations in its substrate binding region. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of subterminally hydroxylated carboxylic acid and P450 monooxygenase variants and mutants, broadly encompassed by the claims. The claims encompass compounds with widely varying structure and properties. However, in this case the disclosure is limited to a method for hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47, 74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. It would require undue experimentation of the skilled artisan to hydroxylate any carboxylic acids.

Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to a method for hydroxylating 15-para-nitrophenoxycarboxylic acids (pNCA), 12-pNCA, 10-pNCA or 8-pNCA with a modified cytochrome P450 monooxygenase of SEQ ID NO:2 having mutations at residue 26, 47,

74, 87, 188 or 354 of SEQ ID NO:2 expressed in a host cell comprising a polynucleotide encoding said modified monooxygenases. It would require undue experimentation of the skilled artisan to make and use the claimed variants and mutants of any P450 monooxygenases. In view of the great breadth of the claim, amount of experimentation required to make the claimed polynucleotides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by this claim.

While enzyme isolation techniques, recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass a method for the enzymatic production of any or all subterminally hydroxylating aliphatic carboxylic acids using any or all mutants and variants of any P450 monooxygenase, because the specification does not establish: (A) regions of the substrate binding region of any P450 monooxygenase which may be modified without

affecting P450 monooxygenase activity or having an altered substrate profile; (B) the general tolerance of P450 monooxygenase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue with an expectation of obtaining the desired biological function; (D) any or all aliphatic carboxylic acids which are subterminally hydroxylated with P450 monooxygenases; (E) a rational and predictable scheme for selecting aliphatic carboxylic acids with an expectation of obtaining a subterminally hydroxylated aliphatic carboxylic acids by incubating said substrates with a modified P450 monooxygenase; and (F) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method for the production of any or all subterminally hydroxylated aliphatic carboxylic acids using any or all variants and mutants of any P450 monooxygenase. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of mutants and variants of any P450 monooxygenase having the desired biological characteristics recited in the claim is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 12 and 14-17 are rejected under 35 U.S.C. 102(b) as being anticipated by Graham-Lorence et al. (form PTO-1449)

Claims 12 and 14-17 are drawn to a method of producing subterminally hydroxylated aliphatic carboxylic acid by reacting C_8-C_{12}-carboxylic acid derivatives with a modified P450 monooxygenase wherein said modification is a mutation at residue 87 inSEQ ID NO:2 and wherein said modified enzymes shows an altered substrate profile. (See rejection of "C_8-C_{12}-carboxylic acid derivatives" under 35 U.S.C. 112, 2nd paragraph).

Graham-Lorence et al. discloses a method for producing subterminally hydroxylated aliphatic carboxylic acids with a modified P450 monooxygenase having a valine residue at position 87 of SEQ ID NO:2, wherein the mutant enzyme shows an altered substrate profile (abstract and page 1129). The method of Graham-Lorence et al. uses the reductant recited in claim 17 (page 1127). Therefore, the teachings of Graham-Lorence et al. anticipate claims 12-14 and 16-17.

In response to the previousOffice Action, applicants have traversed the above rejection. Applicants argue that Graham Lorence et al. does not anticipate the claimed invention because the reference teaches a method of hydroxylating arachidonic acid, which is not an C_8-C_{12} carboxylic acid. Examiner respectfully disagrees. It should be noted that the claims are not limited to hydroxylating C_8-C_{12}-carboxylic acids only but

the claims are drawn to a method of hydroxylating C_8-C_{12}-carboxylic acid derivatives. Arachidonic acid is a derivative of C_8-C_{12}-carboxylic acid. Therefore the rejection has been maintained.

Claims 12 and 16-17 are rejected under 35 U.S.C. 102(b) as being anticipated by Schwaneberg et al.

Claims 12 and 16-17 are drawn to a method of producing subterminally hydroxylated aliphatic carboxylic acid by reacting C_8-C_{12}-carboxylic acid derivatives with a modified P450 monooxygenase wherein said modification is a mutation at residue 87 inSEQ ID NO:2 and wherein said modified enzymes shows an altered substrate profile. (See rejection of "C_8-C_{12}-carboxylic acid derivatives" under 35 U.S.C. 112, 2nd paragraph).

Schwaneberg et al. (form PTO-1449) teach a method for subterminal hydroxylation of pNCA (para-nitrophenoxycarboxylic acids), 6-pNCA, 8-pNCA, 10-pNCA and 11-pNCA with a modified P450 monooxygenase having an alanine residue at position 87 of SEQ ID NO:2, wherein the mutant enzyme shows an altered substrate profile (abstract and pages 359 and 364). The modified P450 monooxygenase of Schwaneberg et al. hydroxylates both terminal and subterminal position of pNCA (Table 1, page 364). The method of Schwaneberg et al. uses the reductant recited in claim 17 (abstract). Therefore, the teachings of Schwaneberg et al. anticipate claims 12-14 and 16-17.

None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned are 571-273-8300 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Yong D. Pak
Patent Examiner 1652

Manjunath Rao
Primary Examiner 1652